REMARKS

Reconsideration is requested.

Claims

The details of claim 66 has been added to claim 63, and claim 66 canceled, to advance prosecution. Claims 76 and 80 have been amended to be dependent on claim 63. Claims 1-62, 66, 81, 82, 87 and 91 have been canceled, without prejudice.

At a minimum, the amendments are believed to obviate the following rejections:

(1) the Section 102 rejection of claims 63-65, 67-73, 75-76 and 78-79 over Pasarell (Journal of Clinical Microbiology, July 1990, pp 1665-1657);

(2) the Section 102 rejection of claims 63-65, 67-68, 73 and 78-81 over Dubeau (Biotechnology Letters, 1987, Vol. 9, No. 4, pp 275-280); and

(3) the Section 102 rejection of claims 63-64, 67, 68, 70-72, 77-78 and 82 over Honbo (Sabouraudia: Journal of Medical and Veterinary Mycology, 1984, 22, 301-310).

The Examiner's indication that the subject matter of dependent claim 66, which included the details of independent claim 63 from which claim 66 depended, is patentable over Pasarell, Dubeau and Honbo is acknowledged, with appreciation. The additional above amendments are believe to place all of the remaining claims in condition to be allowable over at least Pasarell, Dubeau and Honbo. As the amendments will at least overcome these rejections, and hence reduce the issues or any possible appeal, entry of the present Amendment is requested.

The present Amendment is submitted to place all of the claims in condition for allowance over the art of record, for the reasons detailed herein, such that entry of the

present Amendment and allowance of all of the pending claims, including rejoinder and allowance of claims 83-86 and 88-90, are requested.

The Section 102 rejection of claims 63-67 and 72 over Cotty (U.S. Patent No. 5,171,686), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The rejected claims 64-67 and 72 are dependent on independent claim 63. Claims 63 provides an antigenic composition for detecting antibodies from a sample of a test subject, wherein the composition contains a fungal or yeast cell culture supernatant containing fungal or yeast components shed into the supernatant during culturing.

The cited art does not teach such a supernatant. Rather Cotty describes a process of producing strains of highly aggressive *A. flavus* which produce "little or no aflatoxin". See, paragraph spanning columns 1 and 2 of Cotty.

Cotty is concerned with producing compositions which will not contain aflatoxin or, in terms of the presently claimed invention, compositions which would not be antigenic compositions and would not be capable of detecting antibodies from a test subject.

Cotty does not teach a supernatant composition of the presently claimed invention. Cotty identifies isolate origins in Table 1 of Cotty and the examination of isolates "in culture" is described (see, column 3 of Cotty).

Cotty describes "transfer" of colonies to 5/2 agar (5% V-8 vegetable juice, 2% agar, adjusted to pH 5.2 prior to autoclaving) and "utilization" of the colonies directly in the studies of Cotty. Seed isolates were obtained on 5/2 agar either directly from the lint or from pieces of manually delinted seed disinfected for 2 m with 95% ETOH; seed

isolate stock cultures originated from single spores. Fungi were maintained in the dark on 5/2 agar at 25°C. to 30°C.

Strains were further "characterized" by Cotty by seeding conidial suspensions (10 .µl) into centers of Petri dishes (9 cm dia.) containing 30-35 ml Czapek solution agar (CZ) with 3% $NaNO_3$ and incubating at either 25°C., 30°C., or 38° C. for twelve days in the dark. Subsequently, conidia were washed from plates with 95% ETOH and the number of sclerotia per plate was estimated.

Cotty did not produce a composition according to the presently claimed invention.

Diameters of sclerotia produced on CZ with 3% $NaNO_3$ at 30°C. were determined by Cotty by video-image analysis. Conidia were washed from plates with 95% ETOH and sclerotia were dislodged with a spatula and fixed in ethanol: glacial acetic acid (1:1 v/v).

This process also did not produce a composition according to the presently claimed invention.

The Examiner will appreciate that the sclerotia of interest to Cotty are compact or hard masses of mycelium and that the conidia of interest to Cotty are asexual spores of fungus. The sclerotia and conidia of interest to Cotty are not supernatant compositions according to the presently claimed invention.

Example 2 of Cotty describes the wounding of plant cultivars and inoculation of the same with conidia of single strains produced in Example 1 of Cotty. Cotton bolls were produced in Cotty's Example 2.

The aflatoxin analysis of Example 3 of Cotty also fails to produce a composition containing a cell culture supernatant according to the presently claimed invention.

Specifically, the process of Example 3 of Cotty involves pulverizing seed and extraction with an acetone:water (85:15) composition. The mixture was shaken for 15 mins, allowed to set overnight and filtered through Whatman paper. Twenty ml of a zinc acetate, aluminum chloride solution (1.1M $(CH_3 COO)_2$ Zn, 0.04M $AlCl_3$) was added with 80 ml water and 5 g diatomaceous earth to 100 ml filtrate. The mixture was shaken, left to settle for 1 to 2 hr, and passed through Whatman filter paper. Filtrate (100 ml) was extracted twice with 25 ml methylene chloride. Fractions were pooled and concentrated to dryness; residues were solubilized in methylene chloride and aflatoxin B_1 was separated and quantified.

This process of Cotty did not involve production of a cell culture or a cell culture supernatant.

Example 3 of Cotty also describes assessment of in vitro aflatoxin production by seeding conidial suspensions (10 µl) in petri dishes (9 cm dia.) containing 30 to 35 ml of A&M agar. After ten days growth at 30°C. agar cultures were transferred directly to 250 ml jars. This process of Cotty did not produce a cell culture supernatant according to the presently claimed invention.

The transferred agar cultures of Cotty were mixed with 25 ml acetone and shaken for 1 minute (min). Methylene chloride (25 ml) was added, the mixture was shaken for 1 min, filtered through 25 g sodium sulfate and the filtrate evaporated to dryness. This process of Cotty did not produce a cell culture supernatant according to the presently claimed invention.

Residues of the evaporated filtrate according to this process of Cotty were solubilized in methylene chloride for thin layer chromatography (TLC). Extracts and

aflatoxin standards were separated on TLC plates (silica gel 60, 250 .µm thick) by development with diethyl ether-methanol-water (96:3:1, vol/vol/vol) and examined under ultraviolet light. Isolates negative for aflatoxin production in initial tests were grown on three plates containing A&M agar which were combined prior to extraction.

Example 3 of Cotty further describes that quantitative estimates of aflatoxin production in vitro were made using the rapid fluorescence method. Culture tubes containing 5 ml of A&M agar were seeded with 100 .µl spore suspensions containing approximately 100 spores/.µl. After three days incubation at 30°C., agar fluorescence 5 mm beneath the mycelial mat was measured with a scanning densitometer. This process of Cotty did not produce a cell culture supernatant according to the presently claimed invention.

Example 3 of Cotty further provides that aflatoxin was extracted with solvents from representative tubes and quantified by thin-layer chromatography in order to construct a standard curve (fluorescence vs. toxin concentration) for each experiment. This process of Cotty did not produce a cell culture supernatant according to the presently claimed invention.

Example 3 of Cotty also describes that aflatoxin production in liquid fermentation was quantified by terminating fermentations with a volume of acetone equal to the initial volume of medium. This mixture was filtered after two hours, mixed with a volume of water equal to twice the initial volume of medium and extracted twice with 25 ml methylene chloride. The extract was dried and solubilized in methylene chloride for thin layer chromatography as described above. This process of Cotty did not produce a cell culture supernatant according to the presently claimed invention.

Similarly, the remaining procedures of Cotty are not believed to produce a composition of the rejected claims. Withdrawal of the Section 102 rejection of claims 63-67 and 72 over Cotty is requested.

The Section 102 rejection of claims 63-68, 72, 74, 76 and 78-79 over Groopman (U.S. Patent No. 4,859,611), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The rejected claims 64-68, 72, 74, 76 and 78-79 are dependent on independent claim 63. Claims 63 provides an antigenic composition for detecting antibodies from a sample of a test subject, wherein the composition contains a fungal or yeast cell culture supernatant containing fungal or yeast components shed into the supernatant during culturing.

The cited Groopman patent fails to teach the claimed invention.

Specifically, Groopman teaches production of antibodies which bind to aflatoxin. Groopman does not teach or provide an antigenic composition containing a cell culture supernatant according to the presently claimed invention.

Groopman describes conjugation of aflatoxin B_1 (AFB_1) to bovine gamma globulin (BGG) for preparation of an immunogen. See, column 4, lines 25-26 of Groopman. The source of the AFB_1 is not described in Groopman. Groopman does not teach (literally or inherently) or suggest that the AFB_1 of the immunogen preparation method of Groopman is presented, used or prepared in a composition according to the presently claimed invention.

Groopman describes the use of a radio-labelled ^3H-aflatoxin B_1 as a tracer in the design of a detection method. See, Example 1 of Groopman. A method for preparing

the tracer is not provided by Groopman. Groopman does not teach (literally or inherently) or suggest that the radio-labelled ^{3}H-aflatoxin B_1 tracer preparation method of Groopman is presented, used or prepared in a composition according to the presently claimed invention.

Groopman describes, prophetically, the isolation and detection of aflatoxin in vitro from human urine, serum and milk samples. See, column 11, lines 15-21 of Groopman. These compositions of Groopman, to the extent the prophetic descriptions may be considered a teaching in the art, are not compositions according to the presently claimed invention.

Withdrawal of the Section 102 rejection of claims 63-68, 72, 74, 76 and 78-79 over Groopman is requested.

The present Amendment is submitted to place the application in condition for allowance and a Notice to that effect is requested. At a minimum, entry of the Amendment is requested as the above amendments reduce the issues for appeal.

A Notice of Allowance is requested. The Examiner is requested to contact the undersigned in the event anything further is required.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
B. J. Sadoff
Reg. No. 36,663

BJS:
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100